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Original Article

The functional views on response of host rabbit post coronavirus vaccination via ACE2 PET

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Abstract: Molecular imaging can dynamically and quantitatively record the biochemical changes in a systemic view. In this research, SARS-CoV-2 pseudovirus was intramuscularly injected to simulate the vaccination with inactivated virus. New Zealand white rabbits were evaluated with ^{18}F-FDG PET for inflammation and ^{68}Ga-cyc-DX600 PET for ACE2 fluctuation, which were performed before and at 3, 7 and 14 days post injection (d P.I.); furthermore, one rabbit was vaccinated with two cycles with interval of 14 days for a longer period evaluation. Different with the vaccination-induced inflammatory response that was random and individual, ACE2 regulation was systemic and organ-specific: the liver and spleen were of a moderate decrease post injection but rebound at 14 d P.I., while there were a downward trend in heart, testis and bone marrow; besides, similar pattern of ACE2 regulation were recorded after the second injection with a relatively greater volatility. In conclusion, ACE2 PET gave a more comprehensive view on host response post vaccination, hold substantial promise in continuous monitoring of coronavirus vaccine administration and effectiveness.

Keywords: Inflammation, ACE2, molecular imaging, vaccination, host response

Introduction

COVID-19 has been a global disease that leads to a high incidence of infection and mortality. Although the virulence of SARS-CoV-2 tends to decrease, the sequalae of long COVID bring a new challenge to the public health [1-3]. In current situation, coronavirus vaccination was the best approach to prevent the infection and subsequent severe symptoms. Most of the vaccines currently on the market use the prototype or Omicron strain of SARS-CoV-2 as immunogen to develop vaccines, including live attenuated virus vaccines, inactivated virus vaccines, recombinant viral vector vaccines, DNA vaccines, mRNA vaccines, virus-like particle vaccines, and subunit vaccines, hence, confirming vaccines' effectiveness and protection under policy of widespread vaccination were of actual demands [4, 5]. With the prolonged transmission, the virus has evolved from the original strain into multiple mutated strains, and the infectivity and antigenicity have changed with more diversity; to date, more than 96% of new infections worldwide have been identified as Omicron subvariants [6, 7]. The high number of mutations in the viral spike protein might evade antibodies previously induced by infection or vaccination, hence, the development of broad-spectrum vaccines covering diverse strains are also in full swing [8, 9].

The current surveillance of post-vaccination reaction mostly depends on the blood test on antibodies, such as specific IgG and IgM, and neutralizing antibody [10, 11], so as to determine the dosage and opportunity of multiple injections. Besides the primary outcome of neutralizing antibody to block the attachment to host, local inflammation was often observed in the form of swollen lymph nodes and increased FDG uptake, which normally faded away in a few days [12, 13]. In addition to the abnormal glycometabolism, the high uptake of targeted

tracers, such as PSMA-11, DOTATATE and Choline, were often observed in functional imaging of vaccinated population [14], meaning a series of biochemical changes happened post vaccination. Although the relationship with vaccination was explicit, the principle of abnormal uptake was still unclear. For example, immune activation was proposed as a subsequent outcome of vaccination, and there were also a number of studies on the vaccination-related influence on immune-block therapy (IBT). So far, immune-related factors, such as, PD-1/PDL-1 and CD4/CD8 can be visualized by molecular imaging [15-17], providing the tools in revealing vaccination-related immune activation. More interrelated with vaccination, angiotensin converting enzyme 2 (ACE2) has drawn significant concern as a target protein of host cells in SARS-CoV-2 infection, the performance of ACE2 regulation was the focus throughout the course of coronavirus infection; meanwhile, the ACE2 fluctuations post vaccination is still worth exploring for a deeper understanding of the vaccination-related host response [18]. However, the so-far used detection of ACE2 focus on serum ACE2, which is secondary to surface ACE2 of host cells and lack of a comprehensive understanding of whole-body and multivisceral views on ACE2 fluctuations. ACE2-targeted PET imaging would therefore be of profound help in tracking the *in vivo* systemic ACE2 fluctuation. For ACE PET, peptide DX600, an ACE2-specific inhibitor discovered via phage display, was developed as ACE2-specific PET radiotracer by labeling radionuclides [19, 20].

For one side, biochemical reactions post vaccination involved a multiple and dynamic change, for another side, coronavirus vaccine was normally developed in a pressing and long-term clinical needs, therefore, a demand to characterize the changes was proposed in this research, and a series of molecular imaging methods, including ^{18}F-FDG PET for inflammation and ^{68}Ga-cyc-DX600 PET for ACE2 fluctuation [21], were utilized to record the step-wise post-vaccination changes on New Zealand white rabbits, so as to outline a comprehensive view on post-vaccination reaction.

Materials and methods

Animal models and vaccination

The animal experiments were performed under the guidance of the ethics committee of Shanghai Jiangong Hospital (Approval No.: JGEC2022-001). Five New Zealand white rabbits (male, 2.2 kg-2.5 kg, 2 months old) were used in this research. One was used as the control in immunohistochemical analysis; three were vaccinated with one injection; and the last one was vaccinated with two cycles of injection with interval of 14 days.

Coronavirus vaccine was structured based on lentivirus that enveloped with Spike-protein, the SARS-CoV-2 pseudovirus (pSLenti-CMV-EGFP-3xFLAG-WPRE, OBiO Technology (Shanghai) Corp. Ltd., Batch No. OP0812) was used to simulate the vaccination process. 5×10^6 TU pseudovirus was injected into the right upper limb of each abbits (prone position), which were then fed with normal diet in the following 14 or 28 days that covered one or two cycles of vaccination.

Molecular imaging

The scanning protocol was arranged as ^{68}Ga-cyc-DX600 PET and ^{18}F-FDG PET with interval of 10 hours. All rabbits were required to fast for 6 hours before ^{18}F-FDG PET scan. $^{68}Ga^{3+}$ was eluted from a ^{68}Ge/^{68}Ga generator (1.11 GBq, ITG Isotope Technologies Garching GmbH) and cyc-DX600-DOTA precursors were purchased from Shanghai Artery Medical Co. Ltd. ^{68}Ga-cyc-DX600 was synthesized in house as our previous report [20, 21], and ^{18}F-FDG was purchased from Atom Kexing Co. Ltd. All the radiopharmaceuticals were of strict quality control on radiochemical purity. For ^{68}Ga-cyc-DX600 PET, ^{68}Ga-cyc-DX600 was injected as 5.55 MBq/kg and scanned at 90 min post injection; For ^{18}F-FDG PET, ^{18}F-FDG was injected as 7.4 MBq/kg and scanned at 45 min post injection. PET scans were performed before and at 3, 7 and 14 day post injection (d P.I.).

During the scans, rabbits were anesthetized with 3% isoflurane and scanned one by one in a head-in first and prone position. All the scans were performed on a PET/CT scanner (Biograph64, Siemens, Germany) with the below parameters: for CT, tube voltage: 120 kVp; tube current: 35 mA; pitch: 1.0; reconstructed layer thickness: 1 mm; for PET, acquisition of whole-body images was finished in three beds with three minutes per bed position. Regions of interest (ROIs), including injected points, liver, kidneys, heart, bone marrow, brain, testis and muscle, were manually drawn

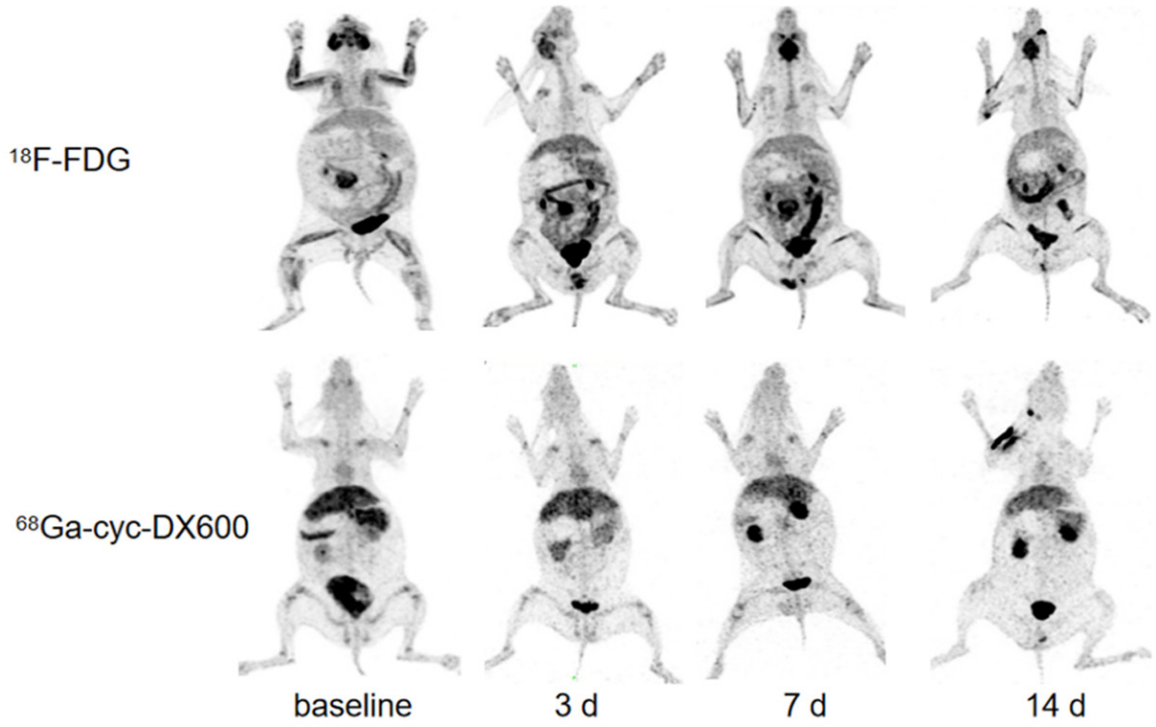

Figure 1. A series of imaging pictures of ^{18}F-FDG PET and ^{68}Ga-cyc-DX600 PET acquired at 4 time points during a cycle of vaccination.

on the transverse section to record the dynamics of SUV_{max}.

Immunohistochemistry analysis

For the primary manifestations by molecular imaging, immunohistochemistry on ACE2 was performed on the main organs that were acquired at 14 d after the one cycle of coronavirus vaccination. For quantification, the integrated optical density (IOD) in IHC results of each organ were analyzed using Image J software, then the correlation analysis between IOD values in IHC and SUV_{max} in PET was conducted.

Statistics

All the data were expressed as mean ± standard deviation, and the paired t-test was used to compare the difference before and after the vaccination. Statistics were performed with SPSS 26, and any difference with *P*-value less than 0.05 was defined as significant.

Results

Molecular imaging gave a more comprehensive and visual understanding on the host response post vaccination (**Figure 1**). Inflammation response in ^{18}F-FDG PET images was random and recovered to a normal level in a short time; furthermore, the most varied findings were systemic ACE2 fluctuation in ^{68}Ga-cyc-DX600 PET.

The changes of main organs post vaccination in ^{18}F-FDG PET and ^{68}Ga-cyc-DX600 PET were respectively displayed in **Figure 2**. For inflammation, only liver and spleen were of increased FDG uptake at 3 d P.I., and recovered to a normal level in a short time; while slight fluctuation were observed in other organs, indicating a prompt and transitory inflammatory response in liver and spleen post vaccination.

Notably, local inflammation response was also detected in ^{18}F-FDG PET during the 14 days observation, manifesting as the injected point in the muscle with high glycometabolism was observed at 3 d P.I. (SUV_{max} = 1.66) (**Figure 3A**) and lasted until 14 d P.I. (SUV_{max} = 0.97) (**Figure 3B**), which disappeared later and did not appear anymore, even in the second cycle of vaccination (**Figure 3C**). The focal FDG uptake induced by local inflammation after vaccination was an individualized and slight phenomenon that should only pay attention in differentiating with lymph nodes-related diseases.

Figure 2. The changes of SUV_{max} of main organs in ^{18}F-FDG PET and ^{68}Ga-cyc-DX600 PET after one cycle of vaccination (before and 3 d P.I., 7 d P.I. and 14 d P.I.).

As the main targets of SARS-CoV-2 infection, ACE2 expression is key for viral entry and replication. For the ACE2 fluctuation, ACE2 PET was intuitive and sensitive to the biochemical apparent changes of ACE2 expression post vaccination (**Figure 2**). Post vaccination administration, ACE2 expression in liver and spleen were of a rebound after a down-regulation process, while there was a downward trend in heart, testis and bone marrow in the observed time window (**Figure 4A**). Additionally, kidneys, as the key components of RAS, were of wide inter-individual variability due to the difference on metabolic rate.

Notably, ACE2 fluctuation were of similar pattern in the second cycle of vaccination. The down-regulation of ACE2 played a key role in blocking the combination between corona virus and host cells. A quick functional compensation on ACE2 expression in liver and spleen potentially due to that angiotensinogen was excreted from liver and then released to blood circulation, while relatively long recovery period was required for heart, testis and bone marrow.

These observation in ^{68}Ga-cyc-DX600 PET were further manifested by IHC on ACE2 at 14 d P.I. (**Figure 4A**), and the linear relationship between immunohistochemistry and PET images further proved the availability of molecular imaging in facilitating the development of vaccine (**Figure 4B**).

Discussion

As proved before by other methods, inflammation, immune activation and ACE2 changes were often detected post vaccination [22]. The design of ACE2-targeted tracer was interesting to scholars due to the epidemics of COVID-19. Indeed, ACE2 was a fluctuated protein in multiple diseases, and the already developed inhibitors, such as DX600 and MLN-4760, have been developed for ACE2 imaging, and utilized in COVID-19 imaging, tumor imaging, and so on. Most tracers were designed on the basis of DX600, which was labeled with Ga-68, Lu-177 and Cu-64 [23]. In addition, the nuclides with more theranostic potentials were being utilized in developing ACE2-targeted agents. There was also the reported tracer developed on the RBD of corona virus [24], but was of the potential risk of inducing the side biochemical reactions. Visualization of vaccine was a promising application of ACE2 imaging, and the reported protocol led no more metabolic burden on host. In this research, the host response post coronavirus vaccination was comprehensively manifested via molecular imaging, mainly focused on the inflammation response and systemic ACE2 fluctuation. Similar to the proved feasibility in revealing post-COVID ACE2 fluctuation [25], ^{68}Ga-cyc-DX600 PET revealed the post-vaccination activation on ACE2, accounting for the optimization of vaccination by a deeper explication of host response. Obviously, the acute dysfunction of ACE2 expression and long-lasting influences should be paid more attentions in vaccine developments.

With the massive injection of coronavirus vaccines in the population, there are increasing reports suggesting focal increased glucose metabolism at injection site in ^{18}F-FDG PET images after vaccination, or accompanied by hypermetabolic lymph nodes in ipsilateral/contralateral axillary, supraclavicular, cervical or mediastinal area, even increased FDG uptake

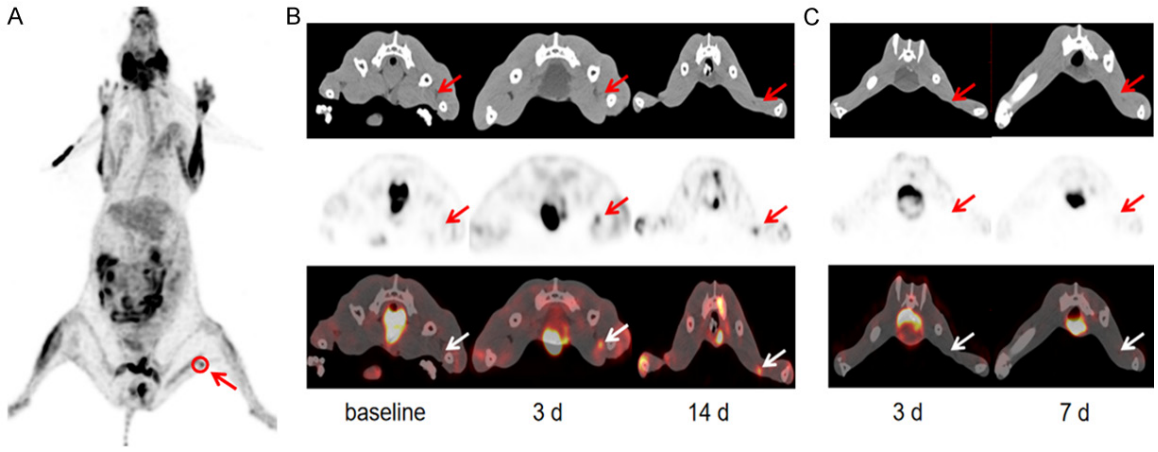

Figure 3. The individual and abnormal findings post vaccination in the images of ^{18}F-FDG PET. Local increased FDG uptake in injected point were observed in MIP images (A), and correspnding cross-sectional images at 3 d P.I. and 14 d P.I. (B); then disappeared in the second cycle of vaccination (C).

in whole spleen [13]. Notably, systemic or local inflammation phenomenon post-vaccination are of individual differences and low incidence in inactivated vaccines, but relatively higher incidence in vaccines with genetic material, such as mRNA vaccine [26]. Vaccine-associated inflammatory responses are mostly mild and can resolve spontaneously; however, for lymph nodes-related diseases, the time window of lymph node response post-vaccine should be considered.

Different with the local and random inflammation at injected points, ACE2 fluctuations were more complex and multi-organs involved, such as heart, liver, spleen and bone marrow. Resulted from the well-known infection process that ACE2 was hijacked as a functional cell receptor for virus attachment and entry by β-coronaviruses, including SARS-CoV-2, the level of ACE2 expression become vital determinants of organ susceptibility. The pattern of ACE2 regulation post vaccination, during coronavirus infection and functional recovery is crucial indicators for monitoring and forecasting. ACE2 PET helps to trace the biochemical process *in vivo*, and gives a comprehensive and timely reflection of pathological changes.

Due to the rapid virus variation of real world, more attention should be paid to hosts that were relatively stable in the virus/host interactions. Vaccine administration is an effective and long-termed strategy for coronavirus infection, for which molecular imaging was of profound help in verifying vaccine effectiveness, evaluating vaccine-induced immunity and monitoring response of host organs. So far, there were five kinds of vaccines in the first line approaches, and hundreds of vaccines were clinically utilized or in pre-clinical phase [27]. Inactivated vaccine was of a relative moderate host response as proved in this study. Among these vaccines, ACE2 fluctuations, including the tensity and period, were key to preventive effects. Therefore, the molecular imaging, especially the imaging protocols with special COVID-related target, were worthy of popularizing in development of vaccines or multiple vaccines protocols. For instance, injection plan of hetero vaccines or the development of vaccine using the dimer of hetero RBD was proposed, and ACE2 PET is therefore of the convenience and advantages to verify the validity of this heterogeneous combination to shorten the cycle of vaccine development.

Conclusion

This research proposed and verified a convenient and flexible imaging protocol, ACE2 PET, in evaluating vaccination by reflecting the host response, so as to facilitate the vaccine development.

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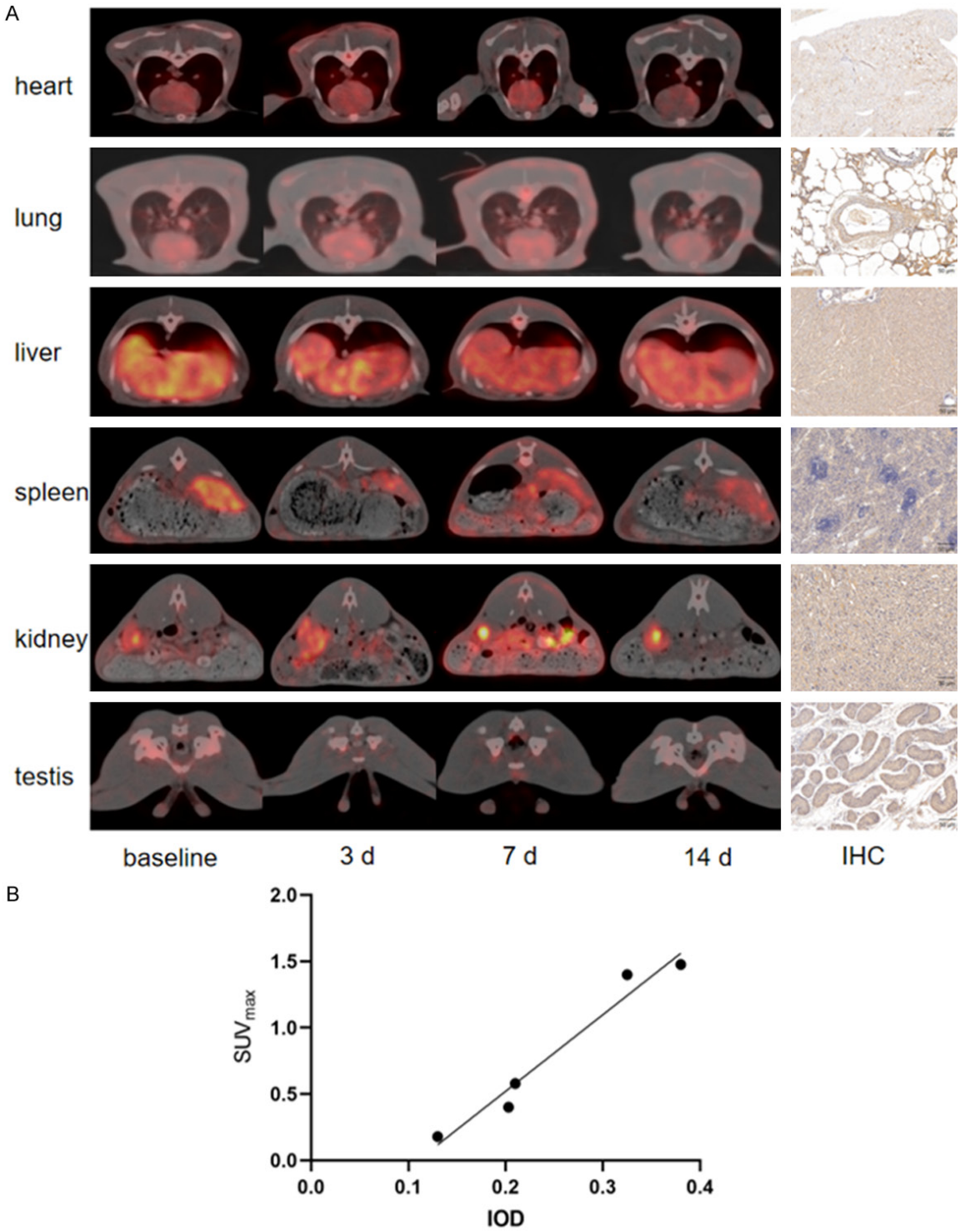

Figure 4. The ACE2 fluctuation in main organs in ^{68}Ga-cyc-DX600 PET post vaccination, and corresponding IHC (20 ×) on ACE2 at 14 d P.I. (A). Correlation between IOD values in IHC and SUV_{max} in ^{68}Ga-cyc-DX600 PET ($r = 0.82$, $P < 0.001$) (B).

Disclosure of conflict of interest

None.

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